10. MILLER, C., F. SKOOG, F. OKUMURA, M. VON SALTZA and F. STRONG 1956. Isolation, structure and synthesis of kinetin, a substance promoting cell division. Jour. Amer. Chem. Soc. 78: 1375–1380.
11. POLLOCK, B. M. and H. O. OLNEY 1959. Studies of the rest period. I. Growth, translocation, and respiratory changes in the embryonic organs of the after-ripening cherry seed. Plant Physiol. 34: 131–142.
12. SCHWARTZ, D. and C. E. BAY 1956. Further studies on the reversal in the seedling height dose curve at very high levels of ionizing radiations. Amer. Naturalist 90: 323–327.
13. SNEDECOR, G. W. 1956. Statistical Methods. (5th ed.) Iowa State College Press, Ames
14. TOOLE, E. H. 1924. The transformations and course of development of germinating maize. Amer. Jour. Bot. 11: 325–350.
15. TOOLE, E. H., S. B. HENDRICKS, H. A. BORTHWICK and V. K. TOOLE 1956. Physiology of seed germination. Ann. Rev. Plant Physiol. 7: 299–324.
16. WOLFF, S. 1954. Some aspects of the chemical protection against radiation damage to *Vicia faba* chromosomes. Genetics 39: 356–364.

INHIBITION OF PHOTOPERIODIC INDUCTION BY 5-FLUOROURACIL[1,2]

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In this paper it will be shown that photoperiodic induction of the cocklebur, a short day plant, is inhibited by the pyrimidine 5-fluorouracil (5-FU). The studies of other workers have shown that 5-FU inhibits the growth of various kinds of cells and tissues by suppressing the formation of thymidine, and that application of thymidine relieves the inhibitory effects of 5-FU. In these cases 5-FU is an inhibitor of DNA synthesis (2, 4, 5). In other cases, however, 5-FU inhibits RNA synthesis. Thus 5-FU inhibits the production of tobacco mosaic viral RNA by tobacco leaves (1). This inhibition, which is relieved neither by thymidine nor by uracil, is associated with incorporation of 5-FU into the viral RNA (3). Inhibition of photoperiodic induction by 5-FU is not reversed by either thymidine or uracil but is reversed by orotic acid (6) which is known to be an intermediate in the biogenesis both of uridine and cytidine and of deoxycytidine and thymidine. Reversal of 5-FU inhibition by orotic acid suggests the hypothesis that the inhibition is related in some way to suppression of nucleic acid metabolism.

The experiments here reported were further designed to determine which component process of floral induction is inhibited by 5-FU. It will be shown that one effect of 5-FU is upon the inductive act by which vegetative buds are so changed that they subsequently develop into floral primordia.

METHODS

The general procedures used in this investigation, which have been described in detail elsewhere (8, 9), were as follows: Cocklebur plants (*Xanthium pennsylvanicum* Wall.[3]) were grown from seed of our standard inbred line. They were maintained in the vegetative condition in the greenhouse by the use of supplementary low intensity light to extend the natural day length to approximately 20 hours per day. The plants were used 60 days or more after planting and, therefore, after the appearance of the first typically mature leaves. One day before each experiment, the plants to be used were sorted according to size of the half-expanded (most sensitive) leaf. One leaf above the half-expanded leaf and all leaves below this were removed from each plant. The plants were then distributed into groups; all groups contained equal representations of each size class of leaf. In general, one group of 10 to 20 plants was used for each treatment of each experiment; each experiment was repeated three to eight times as noted below.

Chemical treatments were applied by dipping the leaf or tip or both into a solution of the chemical in question. Dipping of the apical bud alone in this way results in the retention by the bud of approximately 0.05 cc of treatment solution. Treatment of the

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[3] Plants used have been classified by H. D. Harrington, taxonomist, Colorado State University, using recent manuals of the Illinois region from which the plants originated, as *Xanthium italicum* Moretti. We will continue to use *X. pennsylvanicum* Wall. until the taxonomy of Xanthium has been clarified. Specimens of the plants typical of those used in our experiments are on file in the Colorado State University Herbarium.

TABLE I

DATA RELATING TO EXPERIMENTAL CONDITIONS FOR RESULTS SHOWN IN FIGS 1 TO 6

FIG No.	EXPERIMENT No.	DATE*	PLANTS/ TREATMENT	LEAF LEFT ON PLANT**	No. SIMILAR EXPERIMENTS PERFORMED
1	C-93	2/12/58	10	S-#3	5
2	C-132	4/16/59	20	T-#3	8
3	C-104	5/5/58	13	T-#3	2
4	C-99	3/27/58	15	T-#3	3
5	C-109	5/24/58	10	T-#3	4
6	C-112	7/17/58	20	T-#3	3

* Date plants were placed in cabinets.
** Numbers refer to length of leaf midrib as follows: S-#3, 5.9 to 7.7 cm; T-#3, 6.9 to 8.5 cm.

leaf alone results in the retention of approximately 0.49 cc of solution. The amount of chemical actually transferred to the plant from a given treatment solution is therefore approximately ten times greater when treatment is applied to the leaf than when treatment is applied to the bud. All treatment solutions contained approximately six drops of Tween 20 per liter.

With the exception of the critical dark period experiment (fig 3), a single 16 hour dark period was used for induction. At the end of the dark period, the plants were returned to long day conditions in the greenhouse. Nine days later the apical buds were dissected, examined under a microscope, and classified according to the series of floral stages previously described (7). Floral stage, as used here, is a measure of rate of development and degree of induction of the cocklebur plant. The experimental details of date, leaf size, number of plants per treatment and number of replications of the experiment are given in table I for all experiments here reported.

Standard errors were calculated for the floral stage estimate of each treatment. From the mean treatment standard error, the minimal difference between two treatments required for significance at the 5 % level was calculated and is included in the data for each experiment.

RESULTS

In preliminary experiments it was established that thiouracil and 2,6-diaminopurine are effective in inhibition of photoperiodic induction of the cocklebur. 5-hydroxyuracil (up to 2×10^{-3} M) and 5-mercaptouracil (up to 1×10^{-2} M), kindly supplied by Dr. Thomas J. Bardos, Armour Laboratories, were ineffective in the concentrations used. 5-Fluorouracil (5-FU) kindly supplied by Hoffman-La Roche, Nutley, N. J., is, however, highly effective in this function.

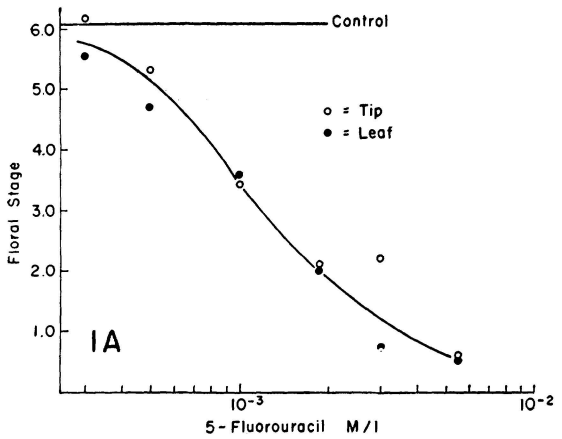
In experiments of which that of figure 1 is typical, 5-FU was applied to cocklebur plants over a wide range of concentrations. Half inhibition of photoperiodic induction, as measured by rate of floral development, is elicited by approximately 10^{-3} M 5-FU. Figure 1 includes data on application of 5-FU to leaves as well as to apical buds alone. In this experiment the concentration of 5-FU which must be ap-

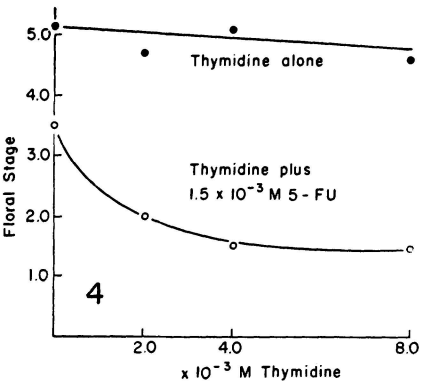
FIG. 1. Inhibition of photoperiodic induction of cocklebur as a function of concentration of applied 5-fluorouracil (5-FU) concentration (A) and amount (B). A single application of inhibitor was made either to the leaf alone or to the apical bud alone. Stage of floral development measured 9 days after the end of the 16 hour inductive dark period. Minimal difference between two treatments required for significance at the 5 % level of probability is 1.41 floral stage units.

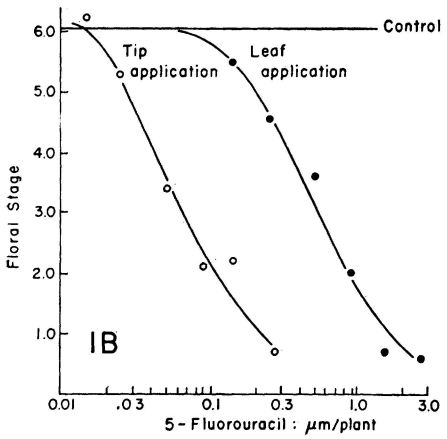
FIG. 2. Inhibition of photoperiodic induction of cocklebur by 5-FU (2×10^{-3} M) as a function of time of application. Stage of floral development measured 9 days after the end of the 16 hour inductive dark period. Minimal difference between two treatments required for significance at the 5 % level of probability is 0.98 floral stage units.

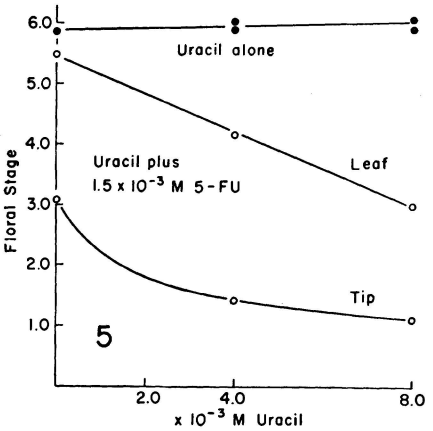
FIG. 3. Inhibition of floral induction of cocklebur by 5-FU (2.0×10^{-3} M) as a function of length of the inductive dark period. A single application of 5-FU was made to both leaf and bud at the start of the inductive dark period in each instance. Stage of floral development measured 9 days after the end of the inductive dark period. Minimal difference between two treatments required for significance at the 5 % level of probability is 0.98 floral stage units.

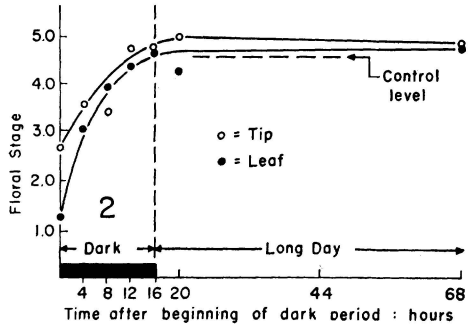
FIGS. 4, 5, and 6. Floral induction of cocklebur as a function of concentration of applied thymidine (fig 4), uracil (fig 5), or orotic acid (fig 6) and in the presence or absence of applied 5-FU. In the experiments of figures 4 and 6 applications were to both bud and leaf, in that of figure 5, to leaf or bud as indicated. Minimum differences between two treatments required for significance at the 5 % level of probability 1.12 (fig 4), 1.15 (fig 5) and 0.98 (fig 6) floral stage units.

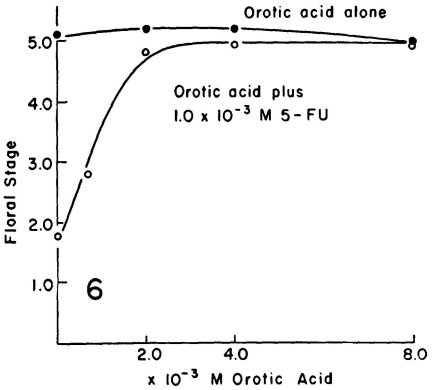


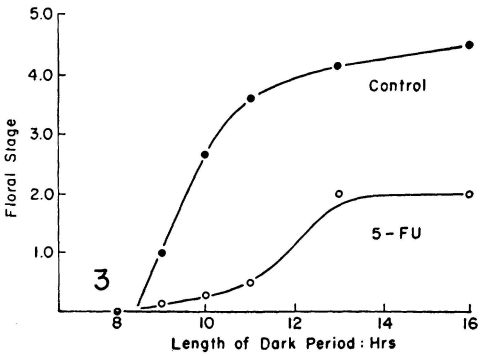












plied to buds to elicit half maximal inhibition is the same as the corresponding concentration for leaf application. The amount of solution and hence of 5-FU applied to the bud is, as noted above, smaller than that applied to the leaf. The 0.05 cc of 10^{-3} M 5-FU solution per bud required to elicit half maximal inhibition, contains 0.05 μ moles of 5-FU. The 0.49 cc of 10^{-3} M 5-FU solution per leaf required to elicit the same effect contains 0.49 μ moles of 5-FU. Thus less 5-FU is required to elicit a given degree of inhibition if the substance is applied to the bud than if it is applied to the leaf.

The greater effectiveness of 5-FU in inhibition of photoperiodic induction, when applied to buds rather than to leaves, was in occasional experiments even more striking than in that of figure 1. In the experiment of figure 5, for example, the application of 5-FU to apical buds produced substantial inhibition in a concentration which was almost ineffective when applied to leaves. The reasons for these variations are unknown.

In the experiment of figure 2, 5-FU was applied to plants at the beginning of, during, or after the end of the inductive dark period. Floral stage, as measured nine days after the inductive dark period, is plotted in figure 2 as a function of time after the beginning of induction. In the experiment of figure 2, as well as in seven others not here presented, 5-FU was most effective when applied at the beginning of the dark period and virtually ineffective when applied at the end of the dark period. This is true for 5-FU application to the tip as well as for application of the material to the leaf. Plants treated with an appropriate concentration of 5-FU at the beginning of the inductive dark period not only do not subsequently flower but continue to grow vegetatively. A single application of 5-FU does not therefore exert any lasting and general inhibition of growth.

A substance which inhibits photoperiodic induction only when it is applied during the dark period may influence either the time-measuring reactions of the dark period or the processes which take place during the inductive dark period but after the expiration of the critical night length (8). Figure 3 shows the results of an experiment designed to determine the effects of 5-FU on the time-measuring phase of the inductive process. Individual groups of cocklebur plants were given single dark periods varying in length from 8 to 16 hours. In figure 3, floral stage 9 days after induction is plotted as a function of length of the dark period. It is clear that in the untreated control plants, flowering took place only when the dark period exceeded approximately 8.5 hours. The same is true for 5-FU treated plants. It is evident, therefore, that 5-FU does not affect the critical dark length and hence does not affect the time-measuring reactions of photoperiodic induction. The data of figure 3 show, however, that 5-FU decreases the effectiveness of the processes which go on in dark periods longer than the critical and which lead to flowering. We may tentatively conclude then that the primary effect of 5-FU upon the act of induction is somehow or other concerned with the floral stimulus which is produced during the inductive dark period.

The data of figures 4, 5, and 6 concern the reversibility of 5-FU inhibition by the nucleotide precursors thymidine, uracil, and orotic acid. It is clear that neither thymidine nor uracil applications reverse 5-FU inhibition of flowering. Indeed, in these experiments combinations of thymidine or uracil with 5-FU caused a greater inhibition of flowering than did 5-FU alone. Neither thymidine, uracil, nor orotic acid by themselves exhibited any effect upon flowering. Orotic acid application does, however, completely overcome the inhibitory effect of 5-FU on flowering (fig 6). This result suggests that the inhibitory effect of 5-FU on flowering may be exerted upon some aspect of nucleotide metabolism.

In all experiments in which 5-FU was applied to cocklebur plants, it was noted that the inhibitor, in addition to suppressing photoperiodic induction, caused a reduction in rate of expansion of the young leaves and elicited some wrinkling of these leaves. It is interesting that orotic acid, which reverses the effect of 5-FU upon flowering, does not reverse the vegetative effects of 5-FU. Thus the effects of 5-FU upon flowering may be separated from those upon vegetative growth.

Discussion

Since 5-FU inhibits flowering effectively only when applied during the single inductive dark period (fig 2) and does not influence the length of the critical night (fig 3), we may tentatively conclude that the effect of 5-FU is upon synthesis or effectiveness of the products of the inductive dark period. From the known interference of 5-FU in nucleic acid metabolism and the inhibition of photoperiodic induction by this compound, we infer that nucleic acid metabolism is involved in the inductive process.

The fact that 5-FU is an effective inhibitor of induction when applied to the apical bud, indicates that an integral part of the inductive process takes place in the bud during the inductive dark period. This has not previously been recognized. It has heretofore been assumed, on good grounds, that the dark reactions are confined to the tissues of the leaf. The present experiments suggest, however, that some portion of the inductive process involving nucleic acid metabolism takes place in the bud during the period of hormone synthesis in the leaf.

Summary

I. Photoperiodic induction of *Xanthium pennsylvanicum*, the cocklebur, is inhibited by application of 5-fluorouracil.

II. A given amount of 5-fluorouracil is in general more effective in inhibiting photoperiodic induction when applied to the buds than when applied to the leaves, the known perceptor organ in photoperiodic induction.

III. 5-Fluorouracil is fully effective in inhibiting photoperiodic induction only if applied during the inductive dark period. This is true even of 5-fluorouracil application to the apical bud. It appears, therefore, that something essential to induction takes place in the bud during the exposure of the leaf to an inductive dark period.

IV. 5-Fluorouracil inhibition of photoperiodic induction is reversed by applying orotic acid. The hypothesis is suggested that photoperiodic induction involves nucleotide metabolism, possibly nucleic acid synthesis.

Acknowledgement

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Literature Cited

1. Davern, C. I. and J. Bonner 1958. The influence of 5-fluorouracil on tobacco mosaic virus production in tobacco leaf discs. Biochem. Biophys. Acta 29: 205–206.
2. Eidinoff, M., J. E. Kroll, and D. Klein 1957. Effect of 5-fluorouracil on the incorporation of precursors into nucleic acid pyrimidines. Arch. Biochem. Biophys. 71: 274–275.
3. Gordon, M. and M. Staehelin 1958. Incorporation of 5-fluorouracil into the nucleic acid of tobacco mosaic virus. Jour. Amer. Chem. Soc. 80: 2340–2341.
4. Heidelberger, C., L. Bosch, N. K. Chaudhuri, and P. B. Danneberg 1957a. Mechanism of action of 5-fluoropyrimidines. Fed. Proc. 16: 194.
5. Heidelberger, C., N. K. Chaudhuri, P. Danneberg, D. Mooren, L. Griesbach, R. Duschinsky, R. Schnitzer, E. Pleven, and J. Scheiner 1957b. Fluorinated pyrimidines, a new class of tumor inhibitory compounds. Nature 179: 663–666.
6. Mitchell, H. K., M. Houlahan, and J. Nyc 1948. The accumulation of orotic acid by a pyrimidineless mutant of Neurospora. Jour. Biol. Chem. 172: 525–529.
7. Salisbury, F. 1955. The dual role of auxin in flowering. Plant Physiol. 30: 327–334.
8. Salisbury, F. 1957. Growth regulators and flowering. I. Survey methods. Plant Physiol. 32: 600–608.
9. Salisbury, F. 1959. Growth regulators and flowering. II. Cobaltous ion. Plant Physiol. 34: 598–604.

EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID APPLICATION ON ACTIVITY AND COMPOSITION OF MITOCHONDRIA FROM SOYBEANS[1, 2]

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It can be assumed that mitochondria possess a normal biological ontogeny; they must have an origin, a period of growth, and lastly, a period of senescence. During growth a series of integrated biochemical syntheses must produce the basic membranous structure as well as the enzymes involved in oxidative phosphorylation. Although biochemical evidence is rapidly accumulating as to the constitutive processes of protein, lipide, and nucleic acid synthesis, there is no description of how these syntheses are controlled and integrated to produce a functional organelle such as the mitochondrion.

The experiments of Lund, et al (12) with sections of corn root of differing mean cell maturity suggest that a significant growth of mitochondria, including a synthesis of oxidative enzymes, occurs during the expansive phase of cell growth. Plant cell expansion is classically known to be controlled by the native growth hormone, or auxin, indoleacetic acid. Although auxins are defined in terms of their capacity to induce cell expansion (24) they are known to produce manifold effects on cellular metabolism, including increases in respiratory rate (1, 3, 21). Auxins, however, have no promotive effect on the activity of isolated mitochondria (3, 17, 23).

Switzer (23) has demonstrated that mitochondria isolated from soybean seedlings sprayed with the synthetic auxin and herbicide 2,4-D[3] exhibit increased oxidative and phosphorylative activity. He concluded that 2,4-D had formative effects leading to the isolation of particles that retained more of their original (in situ) activity. It appears, however, that an equal-

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[2] This investigation was supported in part by Grant G-3358, National Science Foundation, and represents a portion of a thesis submitted by J. L. Key for the Ph.D. degree.

[3] The abbreviations used are: 2,4-D, 2,4-dichlorophenoxyacetic acid; DNP, 2,4-dinitrophenol; RNA, ribonucleic acid; RNase, ribonuclease; AMP, adenosine monophosphate.